



# Immunovirotherapy for the treatment of glioblastoma

## Citation

Cheema, Tooba A, Peter E Fecci, Jianfang Ning, and Samuel D Rabkin. 2014.  
"Immunovirotherapy for the treatment of glioblastoma." *Oncoimmunology* 3 (1): e27218.  
doi:10.4161/onci.27218. <http://dx.doi.org/10.4161/onci.27218>.

## Published Version

doi:10.4161/onci.27218

## Permanent link

<http://nrs.harvard.edu/urn-3:HUL.InstRepos:11879877>

## Terms of Use

This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA>

## Share Your Story

The Harvard community has made this article openly available.  
Please share how this access benefits you. [Submit a story](#).

[Accessibility](#)

# Immunovirotherapy for the treatment of glioblastoma

Tooba A Cheema<sup>1,2</sup>, Peter E Fecci<sup>1</sup>, Jianfang Ning<sup>1</sup>, and Samuel D Rabkin<sup>1,\*</sup>

<sup>1</sup>Brain Tumor Research Center; Department of Neurosurgery; Massachusetts General Hospital and Harvard Medical School; Boston, MA USA;

<sup>2</sup>Momenta Pharmaceuticals; Cambridge, MA USA

**Keywords:** cancer stem cells, glioma, herpes simplex virus, interleukin-12, oncolytic virus, Treg

**Abbreviations:** GBM, glioblastoma; GSC, glioblastoma stem cell; oHSV, oncolytic herpes simplex virus; Treg, regulatory T cell.

We have recently described a new murine model of glioblastoma, generated by the implantation of syngeneic glioblastoma stem cells into immunocompetent mice, that recapitulates the salient histopathological and immunological features of the human disease. We employed this model to demonstrate the multifaceted activity of an oncolytic herpes simplex virus genetically modified to express interleukin-12, G47Δ-IL12.

Glioblastoma (GBM) remains one of the most lethal tumors that patients and oncologists face today. Extensive microinvasion and the limitations proffered by the surrounding normal brain essentially impede complete surgical extirpation. Challenges are similarly met with the employment of standard adjunctive therapies (including temozolomide-based chemotherapy, radiation therapy, and bevacizumab-based immunotherapy), as tumors often comprise an especially heterogeneous mix of terminally differentiated cancer cells and therapy-resistant GBM stem cells (GSCs). These are situated amidst a microenvironment containing aberrant vascular cells, tumor-infiltrating immune cells of various types and stromal cells.<sup>1</sup> Thus, the successful treatment of GBM (which to date has been hardly, if ever, achieved) is likely to necessitate a rational, multifaceted approach targeting malignant cells, GSCs, and the tumor vasculature.

One component of such a multi-modal strategy would be the elicitation of antitumor immune responses. Our immune system, which has evolved under continuous selection to protect us against pathogens, also has a major role in anticancer surveillance,<sup>2</sup> and has recently

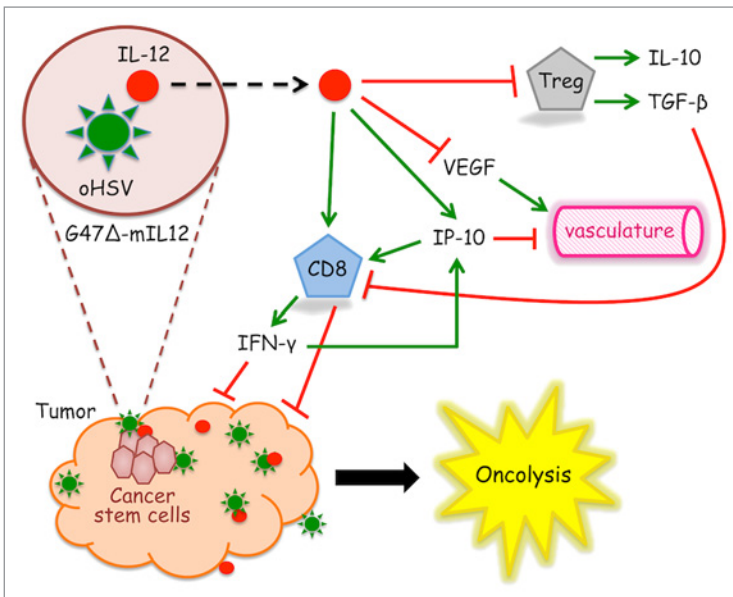
been deployed with increasing rates of success for cancer therapy. The most successful stories told to date in this field include that of sipuleucel-T (Provenge®) and Ipilimumab (Yervoy®), which are now approved by the US Food and Drug Administration for the treatment of prostate cancer and metastatic melanoma, respectively. GBM may also be susceptible to immunotherapy, as glial cells have physiologic antigen-presenting capacities and likewise exhibit some degree of immunogenicity. In support of this notion, GBMs appear to spend a good deal of “energy” to evade the immune system, and recent work has uncovered some of the secrets underlying this process, which involves a subversive shift in T-cell homeostasis toward the accumulation of immunosuppressive regulatory T cells (Tregs).<sup>3</sup> Furthermore, previous concerns related to the immunological privilege of the central nervous system (CNS) have been largely alleviated, as T cells traffic quite well into and out of the brain. Moreover, GBMs generally afford the added “access advantage” given by a myriad of poorly constructed and vascular endothelial growth factor (VEGF)-elicited vessels, which fail to preserve the blood-brain barrier.

We have been studying a rational, multi-modal therapeutic approach against GBM and have employed representative tumor models as an added stringency. In particular, we have recently employed oncolytic viruses “armed” with a cytokine, interleukin-12 (IL-12), that serves not only to promote a T<sub>H</sub>1 immune response, but also to target the vascular microenvironment of GBM while directly killing tumor-initiating GSCs.<sup>4</sup> Oncolytic viruses selectively replicate within (hence killing) cancer cells, but not within their normal counterparts, and thus have the potential to amplify themselves in situ and spread throughout the tumor.<sup>5</sup> Oncolytic herpes simplex virus (oHSV) is particularly well suited for immunovirotherapy because of its inherent cytolytic activity and its ability to induce antitumor immune responses.<sup>6</sup> Furthermore, the third-generation oHSV G47Δ, which is being tested in clinical trials, has the capacity to replicate within and hence kill human GSCs.<sup>7</sup> Immunotherapeutic strategies based on oncolytic viruses that express immunostimulatory cytokines such as granulocyte macrophage colony-stimulating factor (GM-CSF) have already shown efficacy in melanoma

\*Correspondence to: Samuel D Rabkin; E-mail: rabkin@mgh.harvard.edu

Submitted: 11/14/2013; Accepted: 11/15/2013; Published Online: 01/01/2014

Citation: Cheema TA, Fecci PE, Ning J, Rabkin SD. Immunovirotherapy for the treatment of glioblastoma. *OncolImmunology* 2013; 2:e27218; <http://dx.doi.org/10.4161/onci.27218>



**Figure 1.** Multifaceted antineoplastic activity of an interleukin-12-expressing oncolytic herpes simplex virus. G47Δ-mIL12 infects and replicates within malignant cells, including cancer stem cells, hence killing them. As G47Δ-mIL12 amplifies and spreads throughout the tumor, it promotes the expression and release of interleukin-12 (IL-12), which in turn stimulates the production of interferon  $\gamma$  (IFN $\gamma$ ). This leads to the inhibition of angiogenesis upon the upregulation of chemokine (C-X-C motif) ligand 10 (CXCL10, best known as IP-10) and the downregulation of vascular endothelial growth factor (VEGF). IL-12 also inhibits the immunosuppressive functions of regulatory T cells (Tregs) and polarizes antitumor immune responses toward a  $T_H1$  profile dominated by  $CD8^+$  T cells. Thus, G47Δ-mIL12 targets both malignant cells and their microenvironment while stimulating an antitumor immune response.

patients in the context of a Phase III clinical trial (NCT00769704).

To evaluate our immunotherapeutic approach in stringent conditions, we utilized a new murine model of GBM, relying on the orthotopic implantation of 005 GCSs in syngeneic C57Bl/6 mice.<sup>4</sup> The cancer stem cell origin of GBMs in this model is important to properly assess GBM-targeting interventions. Once implanted in the brain, 005 cells form tumors that reproduce the microenvironment and pathology of human GBMs: they are morphologically heterogeneous and include multinucleated giant cells as well as cells that express stem-cell markers (i.e., nestin, prominin), they invade the surrounding brain parenchyma, and they exhibit extensive angiogenesis resulting

in an aberrant vasculature.<sup>4</sup> In addition, these tumors are poorly immunogenic, as malignant cells do not express on their surface MHC class I molecules, CD40 or CD80, and recapitulate the immunosuppressive microenvironment of human GBMs by promoting the accumulation of Tregs.<sup>4</sup>

The intratumoral administration of an IL-12-expressing version of G47Δ (G47Δ-mIL12) effectively targeted neoplastic lesions and their microenvironment in a multi-modal fashion (Fig. 1), de facto improving the survival of mice bearing 005-derived GBMs.<sup>4</sup> G47Δ-mIL12 inhibited neovascularization and the expression of VEGF not only in 005-derived GBMs but also in GBMs derived from human GSCs.<sup>8</sup> In addition,

this treatment promoted the secretion of chemokine (C-X-C motif) ligand 10 (CXCL10, best known as IP-10), curbing the angiogenic fuel for tumor growth, and interferon  $\gamma$  (IFN $\gamma$ ), which invariably favored the elicitation of  $T_H1$  immune responses.<sup>4</sup> Importantly, G47Δ-mIL12 counteracted the accumulation of Tregs within GBM lesions, a primary goal of immunotherapies in general, as this favors T cell-mediated antitumor immune responses. Of note, the efficacy of G47Δ-mIL12 was abrogated in T cell-deficient (athymic) mice, demonstrating the importance of T cells in this therapeutic paradigm as well as the benefits of shifting the tumor microenvironment toward  $T_H1$  immune responses at the expense of Tregs. Interestingly, the survival of immunodeficient mice bearing human, but not mouse, gliomas was extended as a result of the anti-angiogenic activity of G47Δ-mIL12.<sup>8</sup> The use of oHSV as a vehicle for the local delivery of IL-12 appears to be critical, as systemic delivery of IL-12 has proven ineffective at altering the tumor microenvironment, while exhibiting significant toxicity in clinical trials.<sup>9</sup> While triggering promising therapeutic responses, only 20% of GBM-bearing mice treated with G47Δ-mIL12 eventually survived. Thus, further improvements to boost or prolong the  $T_H1$  immune response promoted by G47Δ-mIL12 are required.

Thanks to a new immunocompetent orthotopic model of GSC-driven GBM, we demonstrated the multifaceted oncolytic, anti-angiogenic, and immunostimulatory nature of G47Δ-mIL12 (Fig. 1). Such a multidimensional approach is likely to be critical for the successful treatment of GBM in the context of its complex and often uniquely immunosuppressive milieu.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### References

1. Van Meir EG, Hadjipanayis CG, Norden AD, Shu HK, Wen PY, Olson JJ. Exciting new advances in neuro-oncology: the avenue to a cure for malignant glioma. *CA Cancer J Clin* 2010; 60:166-93; PMID:20445000; <http://dx.doi.org/10.3322/caac.20069>
2. Dunn GP, Fecci PE, Curry WT. Cancer immunotherapy in malignant glioma. *Neurosurgery* 2012; 71:201-22, discussion 222-3; PMID:22353795; <http://dx.doi.org/10.1227/NEU.0b013e31824f840d>
3. Fecci PE, Mitchell DA, Whitesides JF, Xie W, Friedman AH, Archer GE, Herndon JE 2<sup>nd</sup>, Bigner DD, Dranoff G, Sampson JH. Increased regulatory T-cell fraction amidst a diminished CD4 compartment explains cellular immune defects in patients with malignant glioma. *Cancer Res* 2006; 66:3294-302; PMID:16540683; <http://dx.doi.org/10.1158/0008-5472.CAN-05-3773>
4. Cheema TA, Wakimoto H, Fecci PE, Ning J, Kuroda T, Jeyaretna DS, Martuza RL, Rabkin SD. Multifaceted oncolytic virus therapy for glioblastoma in an immunocompetent cancer stem cell model. *Proc Natl Acad Sci U S A* 2013; 110:12006-11; PMID:23754388; <http://dx.doi.org/10.1073/pnas.1307935110>

5. Vacchelli E, Eggermont A, Sautès-Fridman C, Galon J, Zitvogel L, Kroemer G, Galluzzi L. Trial watch: Oncolytic viruses for cancer therapy. *Oncoimmunology* 2013; 2:e24612; PMID:23894720; <http://dx.doi.org/10.4161/onci.24612>
6. Todo T. Active immunotherapy: oncolytic virus therapy using HSV-1. *Adv Exp Med Biol* 2012; 746:178-86; PMID:22639168; [http://dx.doi.org/10.1007/978-1-4614-3146-6\\_14](http://dx.doi.org/10.1007/978-1-4614-3146-6_14)
7. Wakimoto H, Kesari S, Farrell CJ, Curry WT Jr., Zaupa C, Aghi M, Kuroda T, Stemmer-Rachamimov A, Shah K, Liu TC, et al. Human glioblastoma-derived cancer stem cells: establishment of invasive glioma models and treatment with oncolytic herpes simplex virus vectors. *Cancer Res* 2009; 69:3472-81; PMID:19351838; <http://dx.doi.org/10.1158/0008-5472.CAN-08-3886>
8. Zhang W, Fulci G, Wakimoto H, Cheema TA, Buhrman JS, Jeyaretna DS, Stemmer Rachamimov AO, Rabkin SD, Martuza RL. Combination of oncolytic herpes simplex viruses armed with angiostatin and IL-12 enhances antitumor efficacy in human glioblastoma models. *Neoplasia* 2013; 15:591-9; PMID:23730207
9. Del Vecchio M, Bajetta E, Canova S, Lotze MT, Wesa A, Parmiani G, Anichini A. Interleukin-12: biological properties and clinical application. *Clin Cancer Res* 2007; 13:4677-85; PMID:17699845; <http://dx.doi.org/10.1158/1078-0432.CCR-07-0776>